Cold air inhalation and exercise-induced bronchoconstriction in relationship to metacholine bronchial responsiveness: different patterns in asthmatic children and children with other chronic lung diseases

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Cold air inhalation and exercise-induced bronchoconstriction (EIB) have both been used as measures of bronchial responsiveness. Both stimuli are often combined in the Nordic climate. The main objective of the present study was to investigate the climatic influence of cold temperatures upon exercise-induced asthma. The secondary aims were: (a) to assess metacholine bronchial hyper-responsiveness and EIB in children with bronchial asthma (n=32; mean age 10.8 years) compared to children with other chronic lung diseases (CLD) (n=26, mean age 10.1 years); and (b) to assess the influence of cold air inhalation upon EIB in the two groups of children.

Methods used were: (a) the metacholine concentration causing a reduction in FEV₁ of 20% (PC₁₀₂₀), (b) maximum FEV₁ fall (ΔFEV₁) after submaximal treadmill run (EIB test); and (c) ΔFEV₁ after submaximal treadmill run while inhaling cold (−20°C) dry air (CA-EIB test).

Geometric mean PC₁₀₂₀ did not differ significantly between the asthma children (1.28 mg ml⁻¹) and the CLD children (2.90 mg ml⁻¹). In the asthma children, mean ΔFEV₁ after EIB test was 12.8% vs 21.8% after adding cold air (P<0.0001), compared to 5.2 and 7.4%, respectively (P=0.03), in the CLD group. Maximum sensitivity and specificity for the EIB test were 69.8% at a fall in FEV₁ of 6.8%; for the CA-EIB test, 72% at a fall in FEV₁ of 10.2%; and for metacholine provocation, 56% at a PC₁₀₂₀ of 1.5 mg ml⁻¹.

In conclusion, children with bronchial asthma are substantially more sensitive to cold air than children with CLD, and EIB is markedly increased by cold air inhalation in asthmatic children, maintaining the specificity of the EIB test and increasing the sensitivity. The low sensitivity of the EIB test is probably influenced by the use of inhaled steroids. Metacholine inhalation test has less specificity and sensitivity in discriminating asthma from other chronic lung diseases.

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Introduction

Non-specific bronchial hyper-responsiveness (BHR) has been recognized as a main feature of bronchial asthma (1). BHR has been described as a direct and indirect bronchial hyper-responsiveness depending upon the method of measurement (2). The concentration of inhaled nebulized metacholine or histamine causing a fall of 20% in FEV₁ (PC₁₀₂₀) is a measure of direct BHR. The maximum reduction in FEV₁ (ΔFEV₁) after a standardized exercise test or after cold air inhalation represents measures of indirect BHR, as the effect upon lung function is caused by mediator release (2). In a group of asthmatic children, the authors have reported previously a high correlation between PC₁₀₂₀_HISTAMINE (PC₁₀₂₀_H) and ΔFEV₁ after a standardized treadmill run with submaximal load (3). Also, inhalation of cold dry air by isocapnic hyperventilation has been used as a tool for measuring indirect BHR (4), and a high correlation with PC₁₀₂₀_H and PC₁₀₂₀_METACHOLINE (PC₁₀₂₀_M), respectively, has been demonstrated (5,6).

In conclusion, children with bronchial asthma are substantially more sensitive to cold air than children with CLD, and EIB is markedly increased by cold air inhalation in asthmatic children, maintaining the specificity of the EIB test and increasing the sensitivity. The low sensitivity of the EIB test is probably influenced by the use of inhaled steroids. Metacholine inhalation test has less specificity and sensitivity in discriminating asthma from other chronic lung diseases.
Table 1. Demographic characteristics of patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Asthma (n=32)</th>
<th>Chronic lung disease (n=26)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), mean ± SD</td>
<td>10.8 ± 3.0</td>
<td>10.1 ± 2.5</td>
<td>n.s.</td>
</tr>
<tr>
<td>Range</td>
<td>6–16</td>
<td>5–15</td>
<td></td>
</tr>
<tr>
<td>Gender (J/T)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgE (kU/l), geometric mean and geometric 95% C.I.</td>
<td>242.3</td>
<td>77.8</td>
<td></td>
</tr>
<tr>
<td>Atopy (+/−)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animal dander allergy (+/−)</td>
<td>18/14</td>
<td>4/22</td>
<td></td>
</tr>
<tr>
<td>Birch pollen allergy (+/−)</td>
<td>14/18</td>
<td>4/22</td>
<td></td>
</tr>
<tr>
<td>Grass pollen allergy (+/−)</td>
<td>14/18</td>
<td>5/21</td>
<td></td>
</tr>
<tr>
<td>Mite allergy (+/−)</td>
<td>7/25</td>
<td>2/24</td>
<td></td>
</tr>
<tr>
<td>Cladosp. herb. (+/−)</td>
<td>5/27</td>
<td>0/26</td>
<td></td>
</tr>
<tr>
<td>Artemis vulg. (+/−)</td>
<td>4/28</td>
<td>1/25</td>
<td></td>
</tr>
<tr>
<td>Food allergy (+/−)</td>
<td>14/18</td>
<td>4/22</td>
<td></td>
</tr>
<tr>
<td>Treatment, regular</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>5</td>
<td>8</td>
<td>n.s.</td>
</tr>
<tr>
<td>B2-agonists</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>DSCG</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Inhaled steroids</td>
<td>22</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

DSCG, Disodium cromoglycate. To be considered allergic to an allergen, a positive skin prick test of at least ++ (half of the reaction to histamine) or RAST class 2 to the allergen were required.

The effect of adding cold air inhalation to a submaximal run on a treadmill (EIB-test) could improve sensitivity and specificity compared with 

**Patients and Methods**

**PATIENTS**

The subjects of the present study were inpatients at Voksentoppen Centre of Asthma, Allergy and Chronic Lung Diseases, the tertiary referral centre of allergy and lung diseases of children in Norway. The subjects were included consecutively: bronchial asthma, 32 patients (mean age 10.8 years); and other chronic lung diseases (CLD), 26 patients (mean age 10.1 years). Asthma was diagnosed according to the definition given by the International Consensus Report on Diagnosis and Treatment of Asthma (7), and asthma severity was graded by the Kjell Aas score 1–5 (mild–most severe) (8). Two asthma patients were classified as score 1, three patients as score 2, six patients as score 3, 13 patients as score 4, and eight patients as score 5. The group of CLD patients consisted of children suffering from chronic atelectasis (n=1), bronchiectasis (n=5), emphysema (n=1), lobar emphysema (n=3), cystic fibrosis (n=1), chronic sequel after adenovirus pneumonia (n=1) and chronic obstructive non-reversible lung disease (n=14). The latter group was diagnosed by a history of chronic respiratory symptoms and persistent auscultatory findings without the spontaneous variation in lung function and symptoms of the asthma patients, and without reversibility in lung function after inhaled β2-agonist. Table 1 shows demographic data.

**DESIGN**

The tests were performed as a part of the routine clinical evaluation during one stay in Voksentoppen Centre. For safety reasons, the patients always performed the EIB test before the CA-EIB test. The order of performance of PC20-M, exercise test (EIB test) and EIB test with simultaneous cold air inhalation (CA-EIB test) was chosen at random. Intervals of at least 24 h were required between each of the three tests. All measurements were done within a maximum of 10 days. No child had suffered from respiratory infections for the last 4 weeks prior to the study, and all children were free of clinical bronchial obstruction at the time of testing.
METHODS

Lung function was measured by maximally forced expiratory flow-volume curves and by body plethysmograph (Masterlab Body, Erich Jaeger GmbH & Co KG, Würzburg, Germany) at BTPS (body temperature pressure saturated) conditions. Predicted lung function values, when used, were according to Zapletal et al. (9). The variability of baseline FEV₁ measured on two subsequent days, was assessed by use of the coefficient of repeatability (10).

Skin prick test was performed to the Nordic guidelines (11) with the following allergens: moulds (Cladosporium herbarum), house dust mites (Dermatophagoides pteronyssinus), dog dander, cat dander, birch pollen, grass pollen (timothy) and mug worth pollen (Soluprick, ALK, Copenhagen, Denmark). Total IgE was determined by radioimmunosorbent test (Phadebas PRIST, Pharmacia, Uppsala, Sweden), whereas specific IgE was determined against the same allergens as in the skin prick test by the radioallergosorbent test (Phadebas RAST, Pharmacia, Uppsala, Sweden). The results of allergy evaluation are shown in Table 1. To be considered allergic to an allergen, a positive skin prick test of at least ++ (one half of the reaction to histamine 10 mg ml⁻¹) or RAST class 2 to the allergen was required. The patient was considered to be atopic with at least one positive skin prick test or one positive RAST.

Exercise-induced bronchoconstriction (EIB) was determined by running on a motor-driven treadmill for 6 min with submaximal exercise load (EIB test) (12). The inclination of the treadmill was 5.5%, and the speed was adjusted to achieve a steady-state heart rate of at least 180 beats min⁻¹ for the last 4 min of the running time. The heart rate was recorded electronically (Sport-Tester PE 3000 with memory function). FEV₁ was measured before running, immediately after, and 3, 6, 10 and 15 min after cessation of running. Minimum post-exercise FEV₁ at the exercise test was calculated by:

\[
\text{Minimum post-exercise } \text{FEV}_1 = \frac{\text{(pre-exercise } \text{FEV}_1 - \text{minimum post-exercise } \text{FEV}_1)}{\text{pre-exercise } \text{FEV}_1} \times 100\%
\]

Demographic data are shown in Table 1. Age did not differ significantly between the two groups (Table 2). Mean difference between the two baseline measurements of FEV₁ on two consecutive days in all subjects was 0.03 1, corresponding to a mean of 1.8% of the first measurement. Coefficient of repeatability was calculated to be 0.091 s⁻¹, corresponding to 4.8% of the first measurement. Baseline FEV₁ was significantly lower among the CLD patients than among the asthma patients (P=0.03). The other lung function values did not differ significantly between the two groups (Table 2). Mean AFEV₁ after EIB test was significantly greater among the asthma patients (12.8%) than among the CLD patients (5.2%) (P<0.005).

STATISTICAL METHODS

Results are given as mean values with 95% confidence intervals (C.I.) unless otherwise stated. Demographic data are given as mean values ± standard deviation (SD). Differences between the two groups were analysed by the non-parametric Mann-Whitney test. Differences in categorical variables between the asthma and the CLD group were analysed by the χ²-test. Correlation analyses were performed using the Pearson correlation coefficient. All tests were two-tailed with a significance level of 5%.

Baseline FEV₁ was measured on two consecutive days to calculate the coefficient of repeatability, employing the method of Bland and Altman (10). The sensitivity and the specificity of the EIA test, the CA-EIA test and PC_{20,M} in diagnosing asthma from CLD were calculated. The specificity of the test was defined as the proportion of negative responses among the CLD patients correctly identified by the test. The sensitivity of the test was defined as the proportion of positive responses among the asthma patients correctly identified by the test (15).

Results

Demographic data are shown in Table 1. Age did not differ significantly between the asthma group and the CLD group. There were more boys than girls among the asthma patients; the opposite was found in the CLD group. More asthma patients than CLD patients were atopic (P<0.005), and the asthma patients had a higher geometric mean IgE than the CLD patients (P=0.03). Regular treatment with β₂-agonists, disodium cromoglycate and inhaled steroids did not differ significantly between the two groups (Table 1).

Bronchial responsiveness was measured by tidal inhalation for 2 min of each concentration of nebulized metacholine, using doubling concentrations from 0.03 to 32 mg ml⁻¹. A PaRi nebulator with output between 0.13 and 0.16 ml min⁻¹ was used, in the manner described by Hargraves et al. (13) and in accordance with the European guidelines (14). PC_{20,M} was determined as the concentration of metacholine required for a 20% reduction in FEV₁, and determined by linear interpolation on semi-logarithmic curves.

Mean difference between the two baseline measurements of FEV₁ on two consecutive days in all subjects was 0.031 s⁻¹, corresponding to a mean of 1.8% of the first measurement. Coefficient of repeatability was calculated to be 0.091 s⁻¹, corresponding to 4.8% of the first measurement.

Baseline FEV₁ was significantly lower among the CLD patients than among the asthma patients (P=0.03). The other lung function values did not differ significantly between the two groups (Table 2). Mean AFEV₁, after EIB test was significantly greater among the asthma patients (12.8%) than among the CLD patients (5.2%) (P<0.005).
Similarly, the mean reduction in FEV₁ among the asthma patients in the CA-EIB test was 71.8% compared with 7.4% among the CLD patients ($P<0.0001$). Thus, mean ΔFEV₁ was significantly greater after CA-EIB test than after EIB test among the asthma patients ($P<0.0001$), but also among the CLD patients ($P=0.03$). PC$_{20-M}$ did not differ significantly between the asthma patients and the CLD patients (Table 2).

The distribution of the results of PC$_{20-M}$, EIB test and CA-EIB test among the asthma patients and the CLD patients were seen from the scatter plot in Fig. 1. Baseline FEV₁ correlated moderately, but significantly, with log PC$_{20-M}$ in the CLD patients ($r=0.51, P<0.01$), but not in the asthma patients. No significant correlation was found between baseline FEV₁ and ΔFEV₁ after EIB test for the CA-EIB test in either patient group.

ΔFEV₁ correlated significantly with asthma severity score both after EIB test ($r=0.60, P<0.001$) and after CA-EIB test ($r=0.63, P<0.001$). Also, log PC$_{20-M}$ correlated significantly with asthma severity score ($r=0.56, P=0.001$). The relationship between asthma severity score and parameters of BHR is shown in Fig. 2.

A significant correlation for ΔFEV₁ after EIB test and ΔFEV₁ after CA-EIB test was found among the asthma patients ($r=0.86, P<0.001$) and among the CLD patients ($r=0.53, P<0.01$). ΔFEV₁ after CA-EIB test had a significant inverse correlation with log PC$_{20-M}$ in the asthma patients ($r=-0.46, P<0.01$), but not in the CLD patients.

Table 2. Results of baseline lung function measurements, EIB test, CA-EIB test and PC$_{20-M}$ in patients with asthma and patients with other chronic lung diseases

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Asthma (n=32)</th>
<th>CLD (n=26)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline lung function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV₁ (% predicted)</td>
<td>97.3 (92.8-101.7)</td>
<td>86.2 (76.3-96.1)</td>
<td>$P=0.03$</td>
</tr>
<tr>
<td>FEV₁/FVC (%)</td>
<td>80.5 (74.1-86.8)</td>
<td>85.2 (81.7-88.7)</td>
<td>n.s.</td>
</tr>
<tr>
<td>MEF₂₅ (% predicted)</td>
<td>76.9 (70.2-83.7)</td>
<td>80.6 (68.3-93.0)</td>
<td>n.s.</td>
</tr>
<tr>
<td>MEF₅₀ (% predicted)</td>
<td>72.7 (64.5-80.8)</td>
<td>68.0 (56.5-79.5)</td>
<td>n.s.</td>
</tr>
<tr>
<td>VC (% predicted)</td>
<td>94.9 (90.5-99.3)</td>
<td>87.7 (81.4-94.1)</td>
<td>n.s.</td>
</tr>
<tr>
<td>TLC (% predicted)</td>
<td>98.2 (93.3-103.1)*</td>
<td>97.1 (89.3-105.0)†</td>
<td>n.s.</td>
</tr>
<tr>
<td>RV/TLC (%)</td>
<td>42.1 (30.1-54.1)*</td>
<td>34.2 (24.2-44.2)†</td>
<td>n.s.</td>
</tr>
<tr>
<td>SGaw (l kPa⁻¹ s⁻¹)</td>
<td>1.3 (1.1-1.4)*</td>
<td>1.3 (1.0-1.6)†</td>
<td>n.s.</td>
</tr>
<tr>
<td>EIB test, ΔFEV₁ (%)</td>
<td>12.8 (9.5-16.0)</td>
<td>5.2 (3.5-6.9)</td>
<td>$P&lt;0.001$</td>
</tr>
<tr>
<td>CA-EIB test, ΔFEV₁ (%)</td>
<td>21.8 (16.6-26.9)</td>
<td>7.4 (5.0-9.8)</td>
<td>$P&lt;0.0001$</td>
</tr>
<tr>
<td>PC$_{20-M}$ (mg ml⁻¹)†</td>
<td>1.28 (0.79-2.07)</td>
<td>2.90 (1.51-5.57)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Results are given as mean values with 95% confidence intervals (C.I.) in parentheses.

EIB, exercise-induced bronchoconstriction; CA-EIB test, submaximal treadmill run while inhaling cold (-20°C) dry air; CLD, chronic lung diseases; FEV₁, forced expiratory volume in 1 s; ΔFEV₁, maximum fall in FEV₁ after treadmill run with or without cold air inhalation; MEF$_{20-M}$ maximum expiratory flow at 50% of vital capacity; MEF$_{50}$, maximum expiratory flow at remaining 25% of vital capacity; VC, vital capacity; TLC, total lung capacity; RV, residual volume; SGaw, specific airway conductance; PC$_{20-M}$, the dose of inhaled metacholine causing a reduction in FEV₁ of 20%.

*Number of patients (n=28) (asthma patients measured by use of body plethysmograph). †Number of patients (n=20) (CLD patients measured by use of body plethysmograph). ΔFEV₁ in relationship to EIB test and CA-EIB test denotes the maximum change in FEV₁ from before to after the challenge. Values for PC$_{20-M}$ represent geometric mean and antilog of 95% confidence limits of log PC$_{20-M}$ values.

Discussion

The present study demonstrates that children with asthma had greater reduction in lung function after exercise than children with other chronic lung diseases. Furthermore, simulated climatic changes such as cold air influence the
Fig. 1. Scatterplots of PC\textsubscript{20}METACHOLINE (right y-axis), fall in FEV\textsubscript{1} after EIB test (left), and fall in FEV\textsubscript{1} after CA-EIB test (middle) in asthma patients (n=32) and patients with chronic lung disease (n=26). Median values for the different categories are shown with horizontal lines.

Fig. 2. PC\textsubscript{20}METACHOLINE (○), CA-EIB test (△) and EIB test (■) according to asthma severity score (Aas score). The left y-axis denotes fall in FEV\textsubscript{1} (%) after CA-EIB and EIB tests, whereas the right y-axis denotes PC\textsubscript{20}METACHOLINE. The values shown for PC\textsubscript{20}METACHOLINE represent geometric mean for each severity score.

Exercise tolerance to a much larger degree in asthmatic children than in CLD children. Regarding EIB as a measure of indirect BHR, the asthmatic children had a significantly higher BHR than the CLD children, measured both by the standardized EIB test as well as when adding cold air. On the other hand, PC\textsubscript{20}M, being a direct measure of BHR, did not differ significantly between the two groups of children. Thus, the measures of indirect and direct BHR behaved differently between the two groups of children.

BHR has been claimed to be a basic characteristic of bronchial asthma (1) and has been used to separate patients with asthma from healthy subjects (13). Direct measures of BHR as metacholine or histamine inhalation tests have been found useful in identifying asthmatic from healthy subjects (16). However, PC\textsubscript{20}M did not differ significantly between patients with asthma and patients with CLD in the present study. Metacholine-induced bronchial responsiveness had a low maximum sensitivity and specificity, especially in comparison with the CA-EIB test, but also in comparison with the EIB test. These findings are in agreement with the report of Godfrey et al. (17) who found EIB test better suited than metacholine inhalation test to discriminate between asthma and other chronic lung diseases (17). The same group later reported increased sensitivity...
Maximum sensitivity and specificity of the EIB test (68.8%) for discriminating between asthma and CLD were obtained at a reduction in FEV₁ of 6.8% being close to the coefficient of reproducibility of FEV₁ of 4.8%. The corresponding FEV₁ reduction for the CA-EIB test (sensitivity and specificity of 72%) was 10.2%, safer away from the coefficient of reproducibility, thus being a more useful limit. The low sensitivity of the EIB test may partly be explained by the frequent use of inhaled steroids among the study patients. Inhaled steroids in the treatment of childhood asthma affect both direct BHR as measured by metacholine inhalation (19) and indirect BHR as measured by a standardized exercise test (20), and effectively improve EIB in children (20). In the present study, 22 out of 32 children with bronchial asthma were treated with inhaled steroids. Still, as seen from Fig. 1, half of the asthmatic children had \( \Delta \text{FEV}_1 > 10\% \) after exercise, as had 25 out of 32 asthmatic children after exercise and cold air inhalation. This finding, together with the increased reaction to exercise after adding cold air, demonstrates that inhaled steroids do not completely protect against EIB in many asthmatic children, especially not in a cold environment. This underlines the usefulness of exercise testing in asthmatic children as a measure of mastering daily life activities.

Exercise-induced asthma is thought to be elicited by mediator release, provoked by airway cooling (21) and respiratory water loss (22) which subsequently cause a change in osmolality of the pericilliary fluid lining the respiratory tract (23). The respiratory heat and water losses are influenced by the exercise load as well as the temperature and humidity of the inspired air. By use of cold air challenge, a low sensitivity, but a high specificity in identifying asthma patients, has been reported (24). A dose-response relationship has been demonstrated between lung function and cold air challenge until reaching a plateau in lung function (4). Increasing the exercise load (25) or inspiring dry cold air during the exercise may theoretically enhance the magnitude of exercise-induced bronchoconstriction among asthmatic subjects, subsequently increasing the sensitivity of the test. This hypothesis is indeed supported by the present study. Mean AFEV₁ increased from 12.8 to 21.8% in the asthma group by adding dry cold air of -20°C during the exercise. The CA-EIB test, which is easy to perform, seems better suited to discriminate patients with asthma from patients with CLD than both the PC₁₀₀ and the EIB test. The CA-EIB test is especially useful when studying asthma patients treated with inhaled steroids, as it also maintains a high sensitivity during treatment with inhaled steroids.

All measures of BHR in the present study were found to correlate significantly with asthma severity score, thus demonstrating their usefulness in the assessment of asthma severity and in the follow-up of asthma patients.
The EIB test and the CA-EIB test correlated highly in the asthma patients. However, only a low-grade significant correlation was observed between log \( PC_{20-M} \) and the CA-EIB test, and no significant correlation was found with the EIB test in the asthmatic patients. This is in contrast to a previous study by the present authors, where a high correlation between log \( PC_{20-H} \) and EIB test was found. However, only a limited number of the patients in this previous study were treated with inhaled steroids (3), whereas most asthmatic patients in the present study were treated with inhaled steroids. As inhaled steroids have been found to have different effects upon direct bronchial responsiveness (\( PC_{20-M} \)) and indirect BHR (EIB and CA EIB test), it has been suggested that different mechanisms might be involved (20,26). This is further supported by the finding in the present study of a significant correlation between the CA-EIB test and \( PC_{20-M} \) in the asthmatic patients without inhaled steroids in contrast to those with inhaled steroids. The results from the present study suggest that the different measures of BHR reflect different aspects of non-specific BHR, and that different measures of BHR should not be used intermingled.

The present study demonstrates that the magnitude of EIB is increased significantly in low temperatures when inhaling cold air. Inhaled steroids were not found to protect fully against EIB in cold temperature. Furthermore, indirect tests of BHR are more useful in discriminating asthma from other chronic lung diseases than direct tests of BHR such as metacholine inhalation tests. This is especially so when increasing the stimulus of provocation, such as in the present study by combining exercise with inhalation of cold dry air. As physical exercise in cold air represents a commonly encountered stimulus during daily life by many asthmatic children, especially during the winter, adding cold air inhalation to the standardized EIB test at the same time gives information about non-specific bronchial responsiveness, the severity of asthma, and also about the asthmatic child’s exercise tolerance.

### References


